Universidade de Aveiro
Ano 2017
Departamento de Biologia

Vanessa Filipa Santos Rodrigues

Vocalization repertoires variation of Red-billed Chough (*Pyrrhocorax pyrrhocorax*) populations in Portugal

Variação de repertórios de vocalização nas populações de Gralha de Bico Vermelho (*Pyrrhocorax pyrrhocorax*) em Portugal

DECLARAÇÃO

Declaro que este documento é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

Universidade de Aveiro Departamento de Biologia
Ano 2017

Vanessa Filipa Santos Rodrigues

Vocalization repertoires variation of Red-billed Chough (*Pyrrhocorax pyrrhocorax*) populations in Portugal

Variação de repertórios de vocalização nas populações de Gralha de Bico Vermelho (*Pyrrhocorax pyrrhocorax*) em Portugal

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Professor Doutor António Manuel da Silva Luís, Professor Auxiliar no Departamento de Biologia da Universidade de Aveiro, coorientação do Professor Doutor João Alexandre Ferreira Abel dos Santos Cabral, Professor Associado no Departamento de Biologia e Ambiente da Universidade de Trás-os-Montes e Alto Douro, e do Professor Doutor Paulo Jorge Gama Mota, Professor Associado do Departamento de Ciências da Vida da Faculdade de Ciências da Universidade de Coimbra.

o júri

presidente	Professor Doutor Fernando Manuel Raposo Morgado Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro
arguente	Doutor Gonçalo Canelas Cardoso Investigador Pós Doutoramento do Centro de Investigação em Biodiversidade e Recursos Genéticos do CiBio da Universidade do Porto
Co-orientador	Professor Doutor Paulo Jorge Gama Mota Professor Associado da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

agradecimentos

Em primeiro lugar, um profundo agradecimento ao meu orientador, Prof. Doutor António Manuel da Silva Luís, pela orientação, disponibilidade e imprescindível transmissão de conhecimentos quer durante o período de desenvolvimento desta dissertação quer ao longo de todo o meu percurso académico.

Um especial agradecimento aos meus coorientadores, Prof. Doutor João Alexandre Ferreira Abel dos Santos Cabral o e Prof. Doutor Paulo Jorge Gama Mota, pela cooperação e pela indispensável partilha de conhecimento e tempo disponibilizado. Sem a vossa participação a realização desta dissertação multidisciplinar não teria sido possível.

Um especial agradecimento ao Paulo Travassos e à Eliana Soukiazes por todo o tempo que disponibilizaram para me ensinar e ajudar a trabalhar com os equipamentos de som e com o uso do Software para a análise de som.

Gostaria de agradecer ao Sr. Francisco Barros, vigilante da Natureza - ICNF, pela disponibilidade em mostrar os ninhos ocupados pela espécie. Agradeço também ao Francisco Morinha pela disponibilidade.

Finalmente, quero agradecer à minha família, por todo o apoio e amor incondicional e a todos os meus amigos pelo apoio incondicional que me foi dado.

Um muito obrigada a todos.

palavras-chave Aves, Chamamentos, Comportamento Animal, Comunicação Animal, Ecologia.

resumo

As mudanças no estado ecológico dos habitats, induzidas por alterações climáticas e/ou por novos paradigmas dos usos do solo, têm tido inúmeras consequências na distribuição das espécies, desde a fragmentação das populações, passando por mudanças no genoma e no seu comportamento. No caso das aves, estes constrangimentos poderão traduzir-se em mudanças nos repertórios vocais entre populações isoladas. Recentemente, confirmou-se que as duas últimas populações de Gralha de Bico Vermelho (*Pyrrhocorax pyrrhocorax*) residentes em Portugal (populações da Serra d'Aire e Candeeiros e de Sagres) apresentam uma aparente restrição de fluxo genético entre si, sugerindo um isolamento que não se explica apenas pela distância geográfica que as separa. De facto, a espécie apresenta uma elevada capacidade de dispersão o que não pode, só por si, justificar esta restrição de genes. Sendo a espécie um corvídeo com uma elevada capacidade cognitiva e gregária a comunicação entre indivíduos torna-se vital à sua sobrevivência. Este trabalho pretende investigar se a restrição do fluxo genético se deve a uma segregação social que pode refletir-se nas variações do repertório vocal entre as duas populações. Com este intuito, o presente estudo teve como objetivo descrever e tentar compreender quais as principais diferenças entre as duas populações, bem como possíveis variações entre indivíduos de uma mesma população (neste último caso apenas na Serra d'Aire e Candeeiros). Os resultados demonstraram que as duas populações estudadas exibiram repertórios diferentes, com a existência de cinco tipos de vocalizações para a população da Serra na Serra d'Aire e Candeeiros e apenas quatro na população de Sagres, não havendo uma partilha evidente de entre tipos de vocalização comuns a ambas as populações. Verificou-se que entre as populações as maiores diferenças estão na duração, frequência máxima e frequência final, sendo que na população de Sagres as vocalizações têm durações e frequências máximas menores e a frequência final maior que as da população de Serra d'Aire e Candeeiros. No caso das diferenças entre indivíduos analisados ao nível intra-populacional, estes mostraram variações individuais nomeadamente na frequência de pico e do tempo de ascensão. Globalmente, os resultados parecem corroborar a tese de que o isolamento genético destas populações pode ser imputável também a barreiras comunicacionais e sociais.

keywords Animal behaviour, Animal communication, Birds, Calls, Ecology.

abstract Ecological changes in the habitats, induced by climate change or the new paradigms of land use, have had enormous effects in the distribution of species, from the fragmentation of the populations to changes on the genome and/or their behavior. In the case of birds, these constraints may cause changes in vocal repertoires among isolated populations. It was recently confirmed that the two last populations of Red-billed Chough (*Pyrrhocorax pyrrhocorax*) resident in Portugal (populations of the Serra d'Aire e Candeeiros and Sagres) have an apparent gene-flow restriction, suggesting an isolation that is not explained only by the geographical distance that separates them. In fact, the species has a high dispersion capacity which can not itself justify this restriction of genes between these two populations. Being the species a corvid with a high cognitive capacity and gregarious species, the communication between individuals becomes a vital point to its survival. This work intends to investigate if the gene-flow restriction is due to a social segregation that can be reflected in the variations of the vocal repertoire between the two populations. The aim of this study was to describe and try to understand the main differences between the two populations, as well as possible variations among individuals of the same population (in this case, only in Serra d'Aire e Candeeiros). The results showed that the two studied populations have different repertoires, were described five call types from the population in the Serra d'Aire e Candeeiros and only four in the population of Sagres, thus not existing an evident common vocal repertoire between both populations. It was verified that among the populations the greatest differences are in the duration, maximum frequency and final frequency, where the population from Sagres has smaller durations and maximum frequencies and a higher final frequency than the population from Serra d'Aire e Candeeiros. In the case of the differences between individuals analysed at the intra-population level, these showed individual variations, namely peak frequency and ascending time. Overall, the results seem to corroborate the thesis that the genetic isolation of these populations can be attributed to communication and social barriers.

Contents

List of Figures

I – INTRODUCTION

II – MATERIALS AND METHODS

III – RESULTS

LIST OF TABLES

I - INTRODUCTION

Anthropogenic pressures have caused a dramatic change in ecosystems and biodiversity over the last centuries, which are driven by several processes of environmental and landscape changes. Among these, land use/land cover (LU/LC) dynamics are considered major drivers of biodiversity loss worldwide (Foley et al., 2005). In fact, the expected consequences of LU/LC changes are the predicted shifts in species geographic ranges and/or habitat suitability, and the disruption of local biotic communities (Newbold et al., 2015; Titeux et al., 2016). This high levels of disruption drivers in ecosystems, such as rural abandonment, new types of intensive agricultural and agroforestry practices and other human related activities, affects ecological interactions, community composition, populations structure, species reproduction and can even lead to their extinction (Fontúrbel, 2013; Giery et al., 2015).

The ecological and genetic consequences of LU/LC changes is therefore a subject of greatest scientific interest in order to support strategic options for conservation, land use planning and natural resources management (Turner et al., 2007). Some of environmental changes may cause population fragmentation with consequences in their genetic structure/diversity and behaviour, such as communication between individuals. If the communication process is interfered – at the beginning when the signals are produced and transmitted or to the way the signals are received - that interference might cause an impact on reproductive success and consequently on the population viability (Wong, 2014).

The impact of this interference can be enormous for small and isolated populations, which are especially vulnerable to the potential impacts resulting from the ongoing LU/LC changes in their distribution range (Blanco et al. 1997, Morinha et al. 2017a). For example, several bird species learn the social behaviour through vocalizations, such as song dialects, migration routes, tools utilization among others. This may lead to differences in behaviour between fragmented populations (Morinha et al., 2017a).

1.1 Study Species – The Red-billed Chough

a. General Features and Ecological requirements

The Red-billed Chough, *Pyrrhocorax pyrrhocorax*, (Linnaeus, 1758), is a species that belongs to the family of Corvidae. The corvid is medium-sized: around 40 cm and has a

wing-span of 73-90 cm. The species is sexually monomorphic and, as an adult, their body plumage is black, with slight colour variations on the wings and tail; bill and legs are intense red (Figure 1) (Cramp and Perrins, 1994).

Figure 1 – (a) Flock of Red-billed Choughs in Sagres, Portugal. ©Paulo Travassos (b) Couple of Red-billed Choughs in Sagres, Portugal. ©Paulo Travassos

The populations of the Red-billed Chough are highly gregarious and the individuals are monogamous, establishing couples that have a high mate fidelity, being together all year, not just during the breeding season (between April and May). They also have a high nest-side fidelity: the nest occupied by the couple is the same every year, changing only when the local where the nest is located collapses or in the event of widowhood (Banda and Blanco, 2014; Cramp and Perrins, 1994).

Choughs can be found on coastal cliffs or inland rocky mountains, they depend on the existence of caves or abrupt cliffs where they rest or build their nests, which normally have a weak human pressure (Rufino, 1989)**.** However, during feeding time they prefer agriculture or pastures fields, avoing places where the vegetation does not allow them to search actives for invertebrates in the soil (Blanco et al., 1998).

P. pyrrhocorax has a large geographic distribution, from Western Europe to South-East Asia, being classified as least-concern by IUCN (2012)[1]. However its large distribution, the regional populations are small and isolated (Wenzel et al., 2012) that is the reason why the species has a vulnerable status in Portugal (Cabral et al., 2005).

[1] http://www.iucnredlist.org/details/22705916/0 consulted in October 6, 2017 at 21:15

b. Major threats

In Europe, the populations of Red-billed Chough have been decreasing over the last century especially in last three decades of 20th century, this change in numbers of individuals is highly related to intensive agriculture and rural exodus, which consequently drove to the abandonment of traditional farming and pasture (Blanco et al., 1998**;** Morinha et al., 2017a).

Throughout last century the people migrated from rural to urban areas. This trend caused a modification of the countryside landscape, where the uses continuously cultivated, grazed and clean are now abandoned (Cerqueira, 2014). Furthermore, intensive farming being an activity that includes a large use of pesticides and biocides, threatens the life of invertebrates and consequently the all feeding chain of Choughs. Simultaneously, the development of intensive animal farming – since it confines a high number of animals in a limited and covered space – greatly decreases the oxygen rate of the soil, reducing then the amount of choughs' prey (Blanco et al., 1998**;** Mourinha, 2017**;** Oliveira, 1997a).

In opposition, although traditional farming presents similar disadvantages to the ecosystem (nevertheless at a lower scale), choughs have managed to adapt themselves to it and are highly dependent on this kind of agriculture as foraging habitats (Morinha et al., 2017a).

The above modifications on their habitat can endanger small and isolated chough populations, since them have specific foraging requirements and any landscape disturbance can be severe to their viability (Stratford and Stouffer, 1999).

c. Characterization of the Red-billed Chough Populations in Portugal

In birds, factors like low dispersal capacity, geographical barriers and habitat fragmentation can reduce the population diversity of a species. The prevalence of a species relies on the degree of its vulnerability towards environmental changes. That vulnerability may be imprint on their genetic code as a consequence of recent and historic factors, such as geographical and ecological barriers, dispersal capacity and social behaviour (Hoffmann and Willi, 2008; Morinha et al., 2017b; Wenzel et al., 2012)**.**

The populations of Red-billed Chough in Iberian Peninsula have high dispersal capacity where individuals from different populations interact with one another in many regions. The majority of those populations present high levels of genetic diversity, however the peripheral and isolated populations reveal a reduced genetic diversity which can be based on a gene-flow restriction. The Portuguese chough populations are confined to a few and highly isolated regions (Figure 2), which includes mountain habitats of the "Serras de Aire e Candeeiros" Natural Park (Central Portugal) and the "Sudoeste Alentejano e Costa Vicentina" Natural Park (Southwest Portugal, Sagres county), while Spanish populations show an apparent more continuous range (Morinha et al. 2017b). Geographical barriers and low dispersal capacity cannot justify this, which may suggest that social behaviour may be responsible for this genetic restriction (Morinha et al., 2017b).

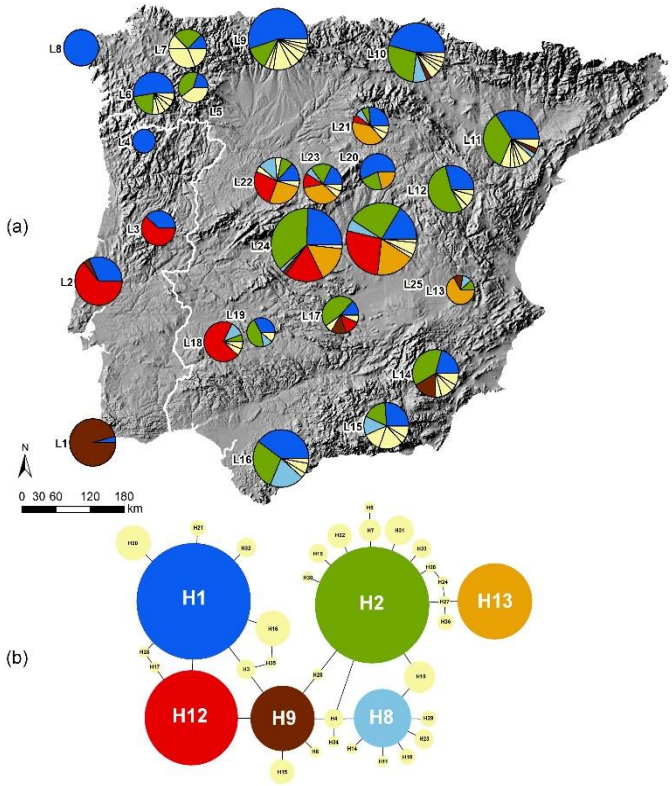

Figure 2 – (a) Distribution of the genetic variation of the nucleus of *P. pyrrhocorax* in Iberian Peninsula. (b) representation of the haplotype network of *P. pyrrhocorax*. Adapted from Morinha et al., 2017b.

Corvids are a good case study for social behaviour in birds, because they are highly gregarious and can recognize other individuals through their calls. This recognition goes

beyond the mates, kin or territorial intruders or neighbours since they can also recognize other individuals from their population, an ability that plays and important role on their social dynamics (Kondo et al., 2010a; Kondo et al., 2010b). Besides the individuals recognition there are species who can recognized individuals from a different origin group, as an example the Killer Whales (Riesch et al., 2006).

The social behaviour as a factor of genetic differentiation is highly studied in mammals – humans, cetaceans, nonhuman primates, elephants and bats – but in birds the studies reflect mostly on recognition of mates, parents and siblings or territorial intruders. Few investigate the recognition of other individuals of their populations (Morinha et al., 2017a; Kondo et al., 2010b).

1.2. Animal Communication

Animals have the need to respond to various external stimuli, whether environmental conditions or from other animals, thus making the communication crucial for almost all the animal species. Communication is the sharing and transmission of information (in form of a signal) by a sender to a receiver, when the signal is sent, the other animal will receive and interpret it (Figure 3). This exchange of information will benefit the sender, but frequently the sender and the receiver have the same interests, this might differ only during a territorial dispute (Bradbury and Vehrencamp, 1998; Catchpole and Slater, 1995).

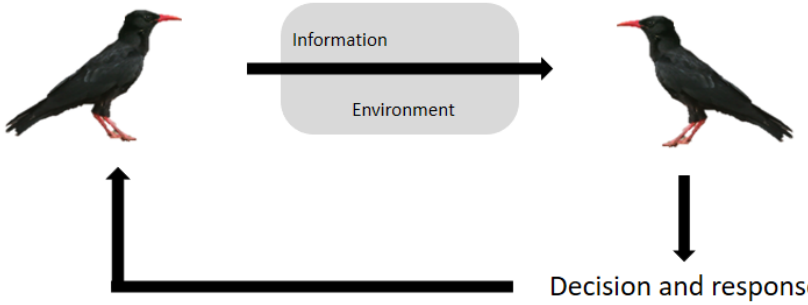

Figure 3 – The process of animal communication. Adapted from Bradbury and Vehrencer 1998.

In animal behaviour/ethological studies it is important to understand the animal communication and, therefore, this theme has received a lot of attention in last decades. By studying the communication of species many relevant information can be extracted (the animal identity, their status or mood, what is their next movement or/and changes on the environment). This information can be passed through electric signals, movements, smells or sounds (Catchpole and Slater, 1995; Kaplan, 2014).

Birds communicate essentially through sound, in general they do not have a developed olfactory system so they develop vision and audition senses. However, depending only on vision would limit their survival capacity, especially during darkness, poor light, in dense habitats and when animals move out of view. Thus, the development of audition diminished those limitations of the sole use of the vision sense. Because sound propagates through matter according to the mass of the object, even when there are visual obstructions, stimuli can still be heard, becoming then a good form of communication (Bluff et al., 2010; Catchpole and Slater, 1995).

Bird vocalizations can be divided in two types: Songs and Calls. Comparing both vocalizations, songs are in general longer, more complex and normally produced by males during the breeding season. On the contrary, calls are shorter, simpler and can be produced by both sexes. Usually, calls happen in specific occasions and functions, such as flight, threat, alarm, and others (Catchpole and Slater, 1995).

a. Vocal repertoire of Red-billed Choughs (P*yrrhocorax pyrrhocorax*)

Every species has its own signal repertoire, even if they are closely related, these different patterns can be a great opportunity to try to understand what made them so diverse, studying the differences in face of the respective environmental contexts (Rosa et al., 2016). The vocal repertoire can change within a species distribution range. In fact, micro and macro-populations can have geographical variants for the same vocalization type, these variations can occur due to environmental or genetic changes, changes in the their learning or a combinations of all these factors (Krebs & Kroodsma, 1980; Laiolo et al., 2001). It is known that the populations form Serra d'Aire e Candeeiros are very isolated, but their vocal repertoire has not been studied yet, so we do not know anything about their vocal

behaviour and whether this may be different. From the distance between the two populations it would be likely that they shared some or all the vocal repertoire (Morinha et al., 2017a).

The number of vocalizations in the Choughs repertoire may vary according to different studies. For example, Laiolo et al. (2000) registered eight calls in all the populations in study. However, Sitasuwa and Thaler (1985) registered a higher number of vocalizations (14 call types), from some individuals in a zoo. These differences on number of calls might have happened because the people who recorded and analysed them used different classification criteria (Laiolo et al., 2000). The people who analysed will also influence an interpretation of each call (Cramp and Perrins, 1994).

Despite the differences between results of the studies mentioned above, there are common specific call types among them. It is well known that Red-billed Choughs vocalize throughout the year, which is not restricted only to the mating season. It was verified the existence of a predominant vocalization, that has several local variants. The others vocalizations are the ones with the greatest difference between populations (Cramp and Perrins, 1994).

The species needs much more research done on its vocal repertoire, especially regarding the geographic variation. Overall, most studies on this variation focus on continuous macro populations, however very few studies have been made taking into consideration the isolated micro populations of Red-billed Choughs (Cramp and Perrins, 1994; Laiolo et al., 2001; Morinha et al., 2017b).

1.3. Objectives

Considering all the LU/LC constraints previously mentioned is important to understand their potential impact on vocal repertoire variations from the last two Red-billed Chough resident populations in Portugal (Serra d'Aire e Candeeiros Natural Park and Sagres), so the objectives of this work are:

1) To describe the vocal repertoire of Chough in both populations;
2) To determine the main differences between populations vocal repertoires;

3) To describe the variation within the repertoire of each individual and among individuals of the same population;
4) To understand if the vocal repertoire variations are related with the apparent gene-flow social segregation between populations.

II – MATERIAL AND METHODS

2.1. Study sites

Two different Red-billed Chough populations were studied in mainland Portugal – Serra d'Aire e Candeeiros Natural Park and Sagres. These two regions are 288.78 Km apart and have the largest resident populations of the species in Portugal – Around 135 individuals in Serra d'Aire e Candeeiros Natural Park and 145 in Sagres (Morinha et al., 2017a).

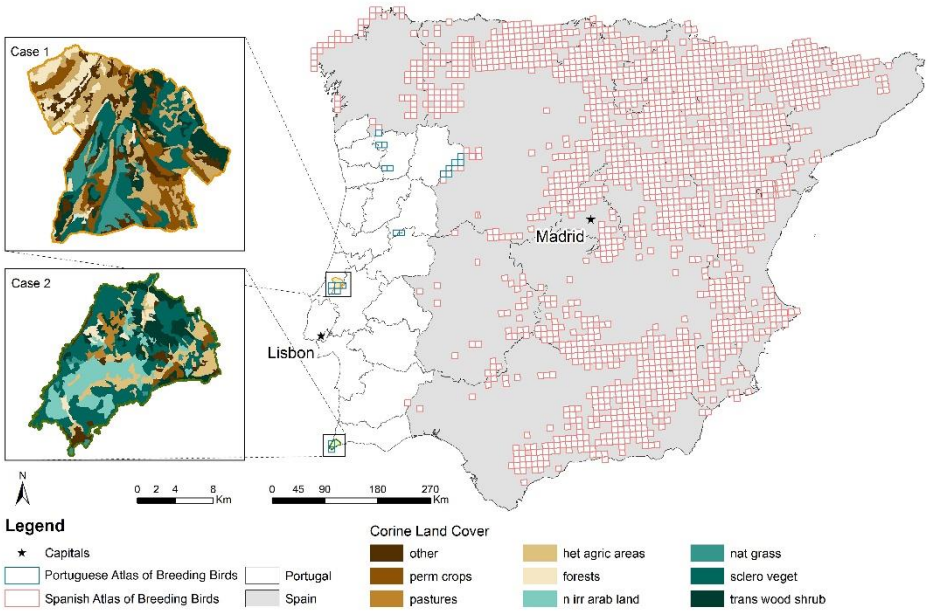

Figure 4 – Representation of the study area. Case 1 is in Porto de Mós (Serra d'Aire e Candeeiros) and Case 2 is in Sagres (adapted from Morinha et a., 2017a).

Serra d'Aire e Candeeiros Natural Park was founded in 1979 and covers an area of 38900 ha, located in Leiria and Santarém districts – centre of Portugal. The Natural Park covers a significant part of the Estremenho Limestone Massif, which belongs to the mountain range "Sistema Montejunto-Estrela". A transition between the Atlantic and the Mediterranean climate, which can be characterized by its dryness and the absence of superficial watercourses. However, the main feature of these mountains is the high number of karstic formations originating caves, algars, and other calcarian structures (Oliveira, 1997b).

Sagres is a village located in Portugal Southeast coast, located in the "Sudoeste Alentejano e Costa Vicentina" Natural ParkThe village is characterized by its sharp cliifs and

irregular track, and the coast is extremelly exposed to the oceanic and atmospheric hard conditions (Loureiro et al., 2008).

Both sites are very windy throughout the year and have a good number of caves and other geological features that can be use as refuges to the Red-billed Chough. In Serra d'Aire e Candeeiros the birds used the algars whereas in Sagres they used the cliifs (Figure 5).

Figure 5 – (a) Main refuge for the population from Serra d'Aire e Candeeiros . ©Paulo Travassos (b) Cave used by a couple of Red-billed Choughs to build their nest, in Serra d'Aire e Candeeiros. ©Paulo Travassos (c),(d) Refuges for the population from Sagres. ©Paulo Travassos

2.2. Recording of calls

The vocalizations were recorded in the field during two different seasons – autumn (Serra d'Aire e Candeeiros in September 28th and 29th and Sagres in October 28th, 29th and 30th) for populations' repertoire characterization and spring (Serra d'Aire e Candeeiros in April 22th and 23th) to determine possible individual vocal signatures.

In autumn, the birds were recorded during early morning, when they left their refuges and then the main group was followed during the day until they came back to the

refuges. In this case, all the calls recorded were considered as individual samples because the birds were not previously identified or ringed.

In spring the method was different and only performed in Serra d'Aire e Candeeiros. The objective was to have several recordings of the same individual in order to find potential "signatures" of individual calls. Several chough breeding pairs were previously identified for recording the call when they left or returned to the respective nests. Since the birds were not identified by a ring, so to identify them it was assign a number to the individual and when the bird appears the number was said in the record, knowing that in mating season the couples of the species are extremely quiet.

Three digital recorders were used – Two Marantz PMD 661 MKII and one Marantz PDM 660 (Marantz, Kanagawa, Japan), each connected to a unidirectional microphone Sennheiser MKH 70 (Sennheiser, Wadermark-Wennenbostel, Germany) with wind protection. In the Spring recordings, an additional microphone was used: a Twin Science MK2 Telinga Parabolic microphone (Telinga, Uppsala, Sweden) (Figure 6).

Figure 6 **–** Recording the population from Sagres using Marantz PMD 661 MKII connected to a unidirectional microphone Sennheiser MKH 70 with wind protection. ©Paulo Travassos

2.3. Sound analysis

The calls were analysed using Avisoft-SASLab Pro (Version 5.2.09, R. Specht, Glienicke, Germany). The recordings were down-sampled to 16000 Hz. To eliminate low-frequency noise a 0.7 kHz high-pass filter was applied with a Hamming window.

Several parameters were measured manually on the calls' spectrograms (table 1 and Figure 7), taken from the fundamental frequency and second/third harmonics. Here,

the one measure would depend on which one had the most energy. At the same time, several possible call types were described and then attributed to each vocalization analysed. Due to the quality of recordings, affected by strong wind or the sound of the sea, some of the parameters, particularly in relation to 2nd and 3rd harmonics could not be measured in a number of calls.

Table 1 - Parameters and their definitions. Based on Kondo et al, 2010b.

PARAMETER	DEFINITION
FI (HZ)	Start frequency of the fundamental frequency.
FASC (HZ)	Ascending frequency is measure between FI and FMAX, but only in calls that have a plateau. The calls that did not have a plateau *were considered* that FASC = FMAX.
FMAX (HZ)	Maximum frequency of the fundamental frequency.
FFIN (HZ)	Final frequency of the fundamental frequency.
FI2 (HZ)	Start frequency of second/third harmonic.
FASC2 (HZ)	Ascending frequency is measure between FI2 and FMAX2, but only in calls that have a plateau. The calls that did not have a plateau a FASC2 = FMAX2 was assumed.
FMAX2 (HZ)	Maximum frequency of second/third harmonic.
FFIN2 (HZ)	Final frequency of second/third harmonic.
TI (S)	Start time of the fundamental frequency.
TASC (S)	Ascending time of fundamental frequency.
TMAX (S)	Maximum time of the fundamental frequency.
TFIN (S)	Final time of the fundamental frequency.
TI2 (S)	Start frequency of second/third harmonic.
TASC2 (S)	Ascending frequency of second/third harmonic.
TMAX2 (S)	Maximum frequency of second/third harmonic.
TFIN2 (S)	Final frequency of second/third harmonic.
DURA (S)	Duration of the fundamental frequency (TFIN–TI).
DURA2 (S)	Duration of the second/third harmonic (TFIN2–TI2).

PKF1 (HZ)	Frequency with maximum sound pressure level on the first harmonic. Peak frequency was measured using the power spectrum.
PKF2 (HZ)	Frequency with maximum sound pressure level of the second or third harmonic. Peak frequency was measured using the power spectrum.
PLATEAU	It is the form of the harmonic – It is the rectangular shape of the call.

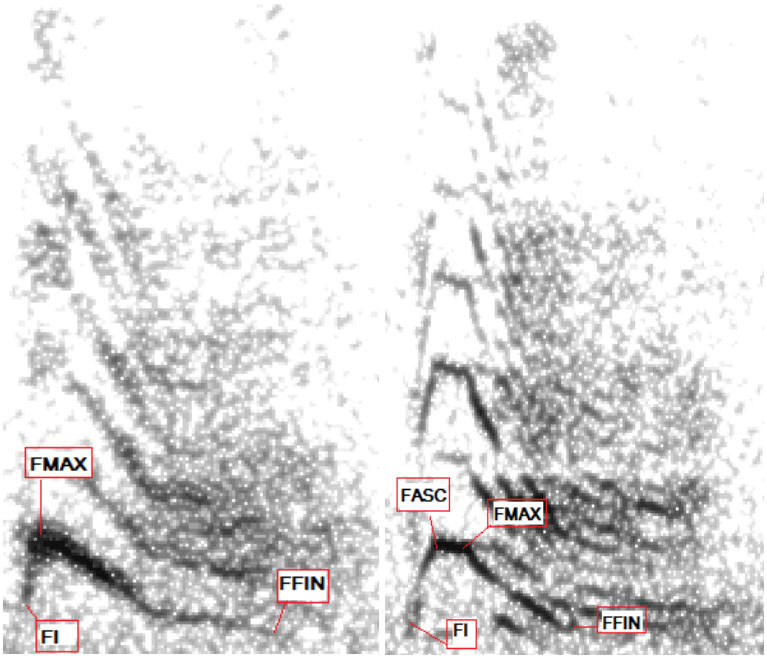

Figure 7 – Spectrogram of a Red-billed Chough that demonstrates how the variables were measured. The image on the left shows a call with no PLATEAU, while the image on right is an example of a call with a PLATEAU.

2.4. Statistical analysis

First, the distribution of variables was analysed in order to detect outliers. To avoid multicolinearity, the variables were tested for correlations with Spearman's rho correlations coefficient (only predictors with correlation lower than 0.7 were selected) (Elith et al., 2006). As a result only seven variables were selected (FI, FMAX, FFIN, FFIN2**,**

DURA, PKF1, PKF2). However, due to the high number of zero values on PKF2 and Ffin2, these two variables were also excluded. The Initial Frequency and Final Frequency were normalized originating Logarithm of Initial Frequency and Logarithm (LogFi) of Final Frequency (LogFfin), respectively.

The remaining parameters were used to perform a discriminant function analysis (DFA). The discriminant function analysis was performed two times, one using the normal method and other with leave one-out method where half of the sample was left out of the analysis. After the DFA, principal component analysis (PCA) was used.

The two PCs were also used to describe and characterized the call type using an ANOVA univariate for the call types from both populations in the PC2 did normalized so it was made an ANOVA One-way.

For the analysis of the individual differences only one call type was considered, in this case call type 1, because it was the most common on single individual recordings. Thus, a total of 30 vocalizations from 5 individuals were analysed by using a ANOVA univariate using for all the variables from the first harmonic. The second and third harmonics were not used due to the high number of zeros. The variables used were the Initial Frequency (Fi), Ascending Time (Tasc), Ascending Frequency (Fasc), Maximum frequency (Fmax), Final Frequency (Ffin), Peak frequency 1 (Pkf1) and Duration (dura).

The statistical analysis was performed using SPSS Statistics (Version 24.0.0.0, IBM Corporation, New York, United States of America).

III- RESULTS

3.1. Description of the vocal repertoire

In order to analyse the differences between vocalization repertoires from the two Red-billed Choughs studied populations, in Serra d'Aire e Candeeiros and Sagres, 568 vocalizations were acoustically recorded and analysed, 201 from Serra D'Aire and 367 from Sagres.

a. Serra d'Aire e Candeeiros Natural Park

After analysis by visual inspection, it was considered that the chough population in Serra d'Aire e Candeeiros exhibited 5 different call types:

Call type 1 (Figure 8) – Most of the calls recorded are of this type (Figure 17); it is a modulated sound with, a fast rise and a slower downward. Several harmonics are visible.

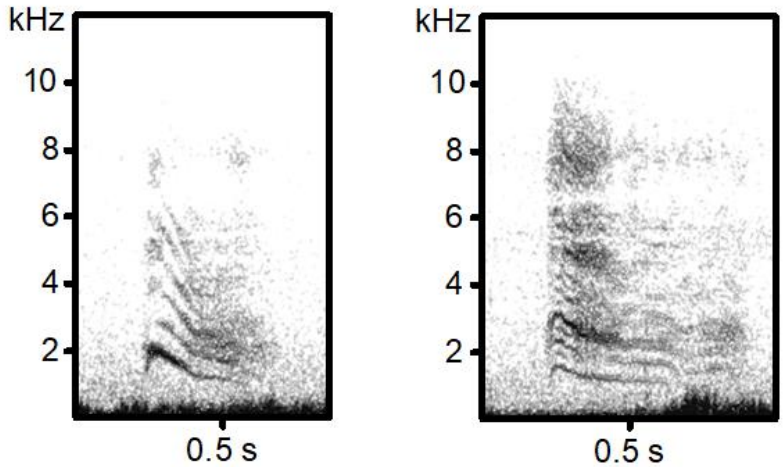

Figure 8 - Spectrograms of the Call type 1.

Call type 2 (Figure 9) – This sound has a high rise, but unlike in type 1, in this call the maximum frequency is maintained or varies little for a few fractions of a second, forming a plateau. There are variants of this call that have some details at the end. Several harmonics are visible.

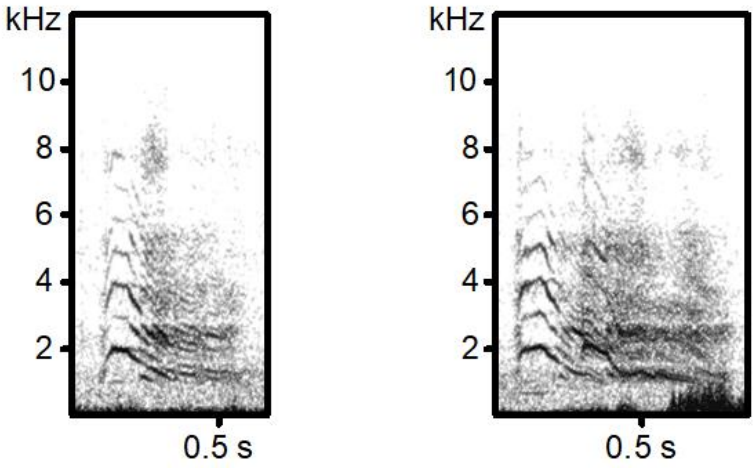

Figure 9 - Spectrograms of the call type 2.

Call type 3 (Figure 10)– It is similar to call type 1; however, its maximum frequency is lower. The downward phase is long but not so uniform as the type 1.

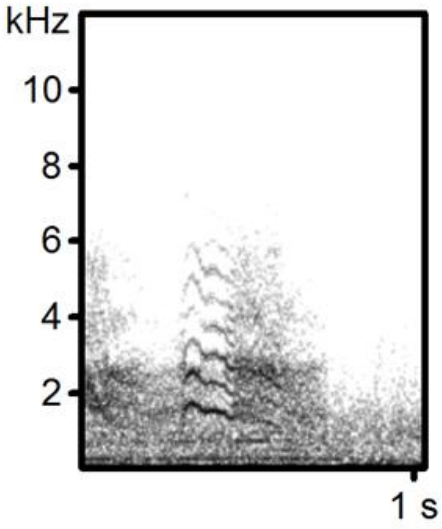

Figure 10 - Spectrogram of Call type 3.

Call type 4 (Figure 11) – The call has a very fast ascending time, with a slower descending phase. This call also has details at the end of the sound; there are variants that also have these details at the beginning of the call. Several harmonics are visible, and particularly complex elements by the end of the call.

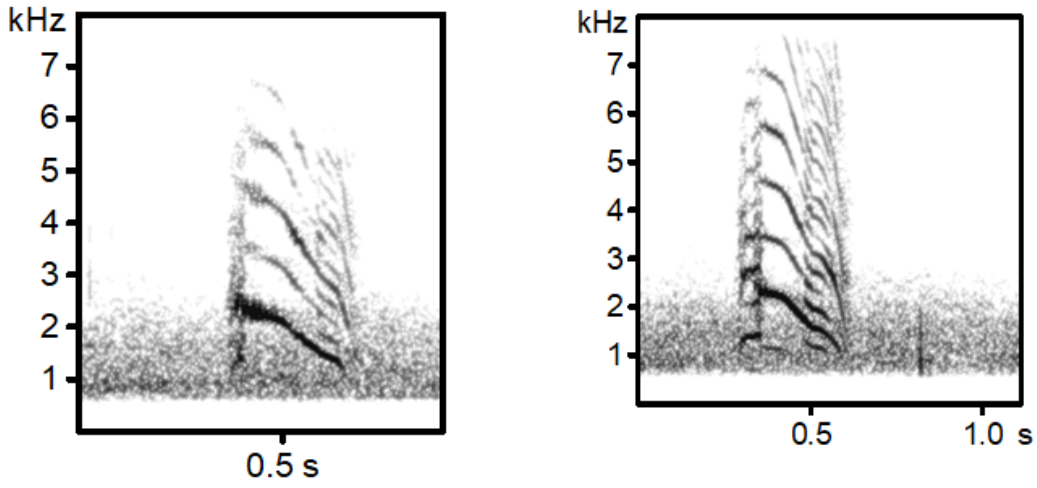

Figure 11 - Spectrograms of the Call Type 4.

Call type 5 (Figure 12) – it is a rare call; It is similar to the first call type but the ascension and the decrease in frequency are more uniform. Does not have an accentuated maximum frequency. Several harmonics are visible.

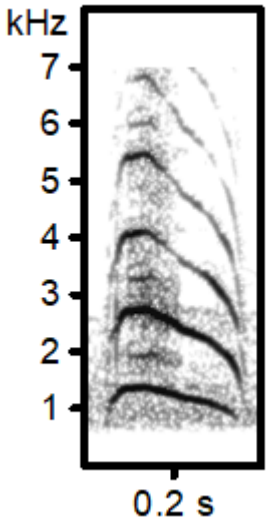

Figure 12 - Spectrogram of the Call Type 5.

b. Sagres

Regarding the Sagres chough population, the vocal repertoire analysed was considered to be composed by 4 different call types:

Call type 6 (Figure 13) **-** It is the most common call registered in Sagres (Figure 17). it is a modulated sound with, a fast rise and a slower downward. It is the equivalent to call type 1 from Serra d'Aire e dos Candeeiros Natural Park, but smaller. Several harmonics are visible

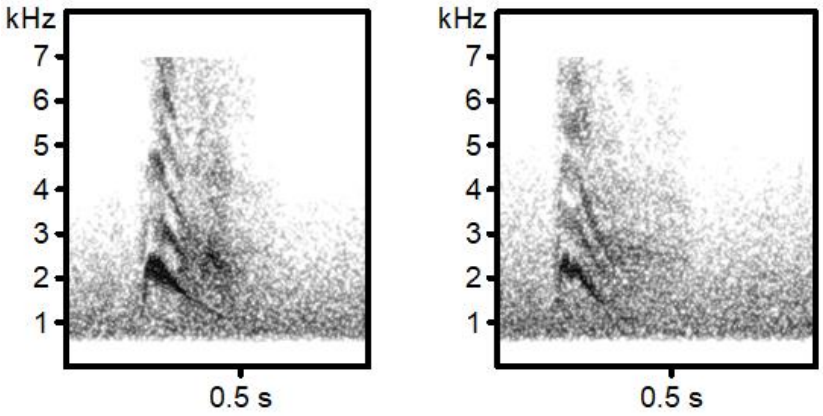

Figure 13 - Spectrograms of the Call Type 6.

Call type 7 (Figure 14) – It is similar to call type 6, but in this call the ascending and descending times are more similar. Several harmonics are visible.

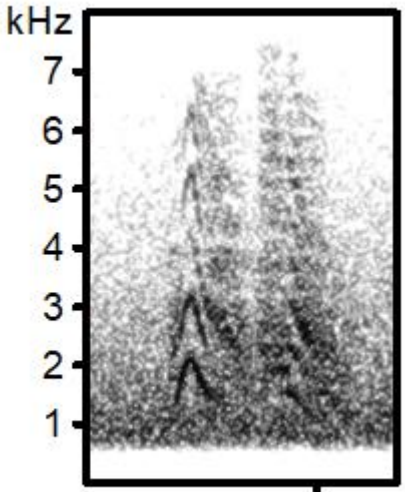

Figure 14 - Spectrogram of the Call Type 7.

Call type 8 (Figure 15)- These calls have a plateau, having a different ascending and maximum frequency, similar to call type 2 from Serra d'Aire, however it is smaller and do not have details at the end of the sound. Several but fewer harmonics are visible.

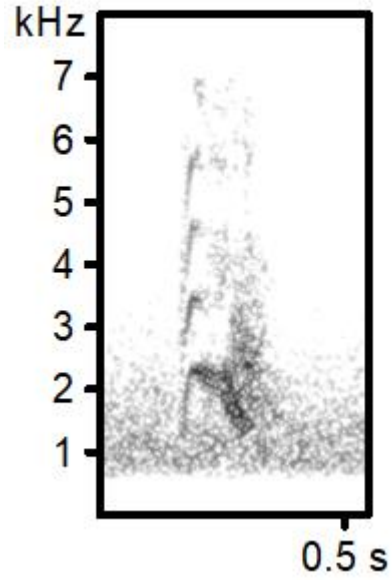

Figure 15 - Spectrogram of the Call Type 8.

Call type 9 (Figure 16) – This call is also similar to call type 6, the only fact that distinguish both calls are the details at the end of the call. Few harmonics.

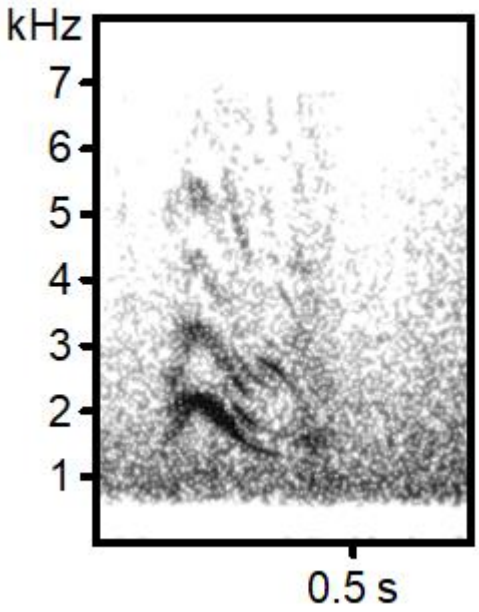

Figure 16 - Spectrogram of the Call Type 9.

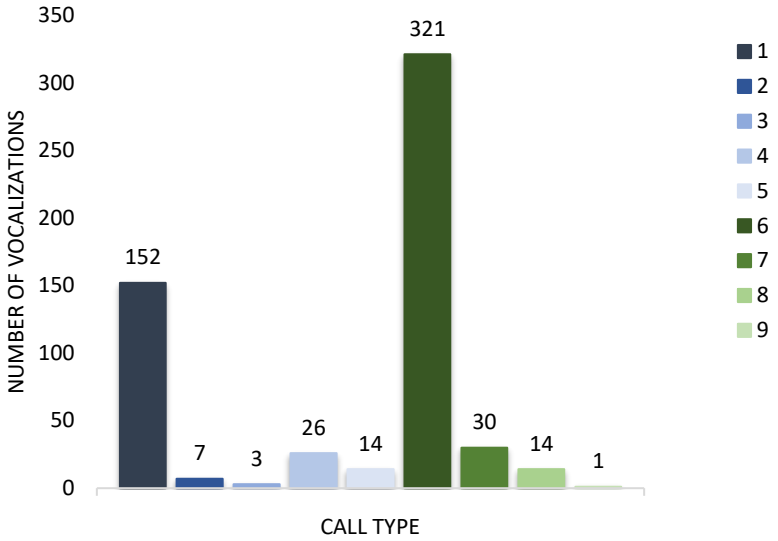

Figure 17 – Representation of the most used call in both populations. The blue colors represent the population from Serra d'Aire e Candeeiros, while the green colors represent the call types used by the population from Sagres.

3.2. Quantitative Characterization of Calls

a. Repertoires of Populations

The discriminant analysis was made assuming the following five variables: 1) peak frequency 1 (Pkf1), 2) Maximum frequency (Fmax), 3) duration (dura), 4) logarithm of the initial frequency (LogFi) and 5) logarithm of final frequency (LogFfin). There are 549 valid samples and 20 are exclude for missing values.

Table II presents the characterization of variables for all calls in the two populations. Peak frequency 1, maximum frequency and duration have lower values in Serra d'Aire population, while initial and final frequencies are slightly lower in Sagres population.

Duration, logarithm of final frequency and logarithm of initial frequency (Table III) are significantly different between populations ($p<0.0001$). The maximum frequency presents a non-significant tendency ($p=0.085$), while there were no differences in peak frequency between populations.

Table II – General statistical description of the five variables for both populations. The Tables shows the mean and std. deviation of the variables for both populations.

	Serra d'Aire	**Sagres**
	Mean (Std. Error)	**Mean (Std. Error)**
Pkf1 (Hz)	2010,05 (22,742)	2029,64 (15,52)
Fmax (Hz)	2334,19 (22,22)	2404,63 (13,27)
Duration (s)	0,264 (0,005)	0,183 (0.004)
LogFi	3.16 (0,006)	3,14 (0,004)
LogFfin	3.01 (0,005)	3,07 (0,004)

Table III – Tests of Equality of Group Means.

	Wilks' Lambda	**F**	**df1**	**df2**	**Sig.**
Pkf1	,999	,628	1	547	,428
Fmax	,995	2,98	1	547	,085
dura	,797	139,46	1	547	,000
LogFi	,991	5,15	1	547	,024
LogFfin	,850	96,22	1	547	,000

i. Comparison of Populations

One discriminant function (DF) that discriminates the two populations was extracted ($p<0.0001$) (Table IV and V). The variable that mostly contributed to the DF is the duration (Figure 18), followed by logarithm of final frequency and logarithm of the initial frequency. The maximum frequency is the variable that contributed the least to the discriminant function (Table VI and VII).

Table IV – Canonical function statistics.

a. First 1 canonical discriminant functions were used in the analysis

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	,411[a]	100,0	100,0	,540

Table V – Statistical significance of the canonical function (Wilks' Lambda).

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	,708	187,83	4	,000

Table VI – Statistical significance of the variables on the canonical function (Wilks' Lambda). a. Standardized Canonical Discriminant Function Coefficients

	Function 1
Fmax	-,230a
dura	,680a
LogFi	,586a
LogFfin	-,535a

Table VII – Centroids of each local under analysis.

Local	Function 1
Serra d'Aire	,891
Sagres	-,460

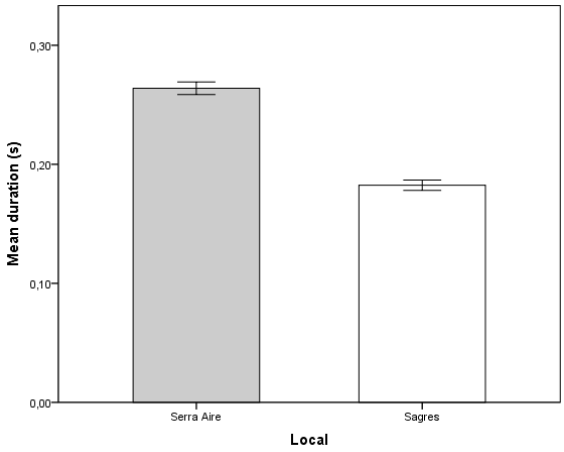

Figura 18 – Duration mean (± standard error) for both populations. Mean from Serra Aire 0,264 (±0,005) and for Sagres 0,183 (±0,004).

Table VIII presents the percentage of calls that were correctly classified in the groups, after being left out for classification. The classification reveals that 73% of vocalizations from Serra d'Aire were correctly classified as well as 77,2% of vocalizations from Sagres, with an average of 75,7%. This percentage indicates that the discriminant function is robust.

Table VIII – Classification results[a,c] of the cases for each local.

a. 75,7% of original grouped cases correctly classified.
b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
c. 75,7% of cross-validated grouped cases correctly classified.

		Local	Predicted Group Membership		Total
			Serra Aire	Sagres	
Original	**Count**	**Serra Aire**	146	54	200
		Sagres	83	281	364
	%	**Serra Aire**	**73,0**	27,0	100,0
		Sagres	22,8	**77,2**	100,0
Cross-validated[b]	**Count**	**Serra Aire**	146	54	200
		Sagres	83	281	364
	%	**Serra Aire**	**73,0**	27,0	100,0
		Sagres	22,8	**77,2**	100,0

A PCA was performed to assess how the variables could be aggregated into principal components (PCs) and how the calls of the two populations varied in relation to the PCs. The PCA extracted two Principal Components (Table IX) with PC1 having higher loadings from the duration and the logarithm of final frequency and PC2 having higher loadings from the logarithm of final frequency and maximum frequency (Table X). The distribution of calls from the two populations by the two principal components (Figure 19) reveals that there is a partial overlap between the two populations, with non-overlapping areas, where the Serra d'Aire population is located on top and left of the distribution while the Sagres population is at the basis and in right side of the distribution. These outputs indicate that the calls from Sera d'Aire are longer, have a higher maximum frequency and a smaller logarithm of final frequency than the calls from Sagres.

Table IX – Principal Component Analysis Factors description.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1,99	39,86	39,86	1,99	39,86	39,86
2	1,39	27,70	67,55	1,39	27,70	67,55
3	,772	15,44	82,99			
4	,448	8,96	91,95			
5	,402	8,05	100,0			

Table X – Component Matrix[a].

a. 2 components extracted.

	Component	
	1	**2**
Fmax	,538	,679
dura	-,637	,564
LogFi	,626	,014
LogFfin	,692	-,546
Pkf1	,653	,555

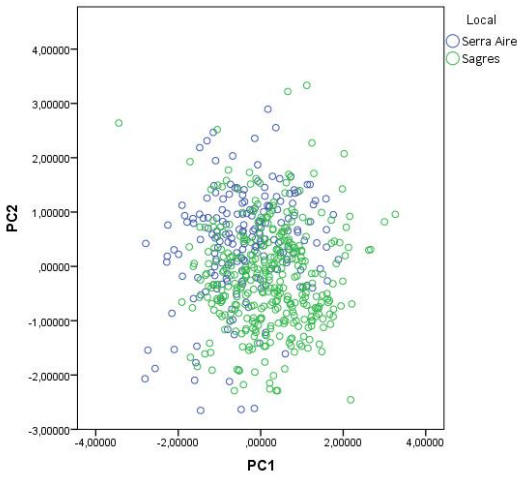

Figure 19 – Representation of the distribution of vocalizations of the two populations in function of the two principal components (PC1 and PC2) of the PCA.

ii. Determination of Call Type Identity

In order to confirm that the previously defined calls are indeed different, and we can consider them as different call types, the two PCs from principal component analysis were tested for the call types that presented more similarities form both populations. We first compared the two most common call types used (, -i.e., call types 1 and 6). The ANOVA reveals that the two call types are significantly different (PC1: p<0,0001 and PC2: p<0,0001).

Table XI – Test of the similarity between Call type 1 and 6, using PC1 as dependent variable.

a. R Squared = ,098 (Adjusted R Squared = ,096)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	44,02[a]	1	44,02	49,69	,000
Intercept	8,6	1	8,6	9,71	,002
Call type	44,02	1	44,0	49,69	,000
Error	404,8	457	,886		
Total	449,00	459			
Corrected Total	448,81	458			

Table XII – Test of the similarity between Call type 1 and 6, using PC2 as dependent variable.

a. R Squared = ,068 (Adjusted R Squared = ,066)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29,26[a]	1	29,26	33,34	,000
Intercept	8,71	1	8,71	9,92	,002
Call Type	29,26	1	29,26	33,34	,000
Error	401,11	457	,878		
Total	431,3	459			
Corrected Total	430,37	458			

A similar approach was used regarding the classification of call types 6, 7 and 8, that had some visual similarity. The ANOVA at the PC1 (Table XIII and Table XIV) demonstrated that call types 7 – 8, and 6 – 8 are significantly different. On the other hand, PC2 (Table XIII and Table XV) shows that call type 6 are significantly different from call type 7, which means

that call type 7 and 8 differ in all variables in analysis as well as call types 6 and 8. The call types 6 and 7 also differ in all variables except logarithm of Initial Frequency (Table X).

Table XIII – Relation between Call type 6, 7 and 8.

			PC1	PC2
	(I) Call type	(J) Call type	Sig.	Sig.
Scheffe	**6,00**	**7,00**	,101	,000
		8,00	,292	,306
	7,00	**6,00**	,101	,000
		8,00	,035	,308
	8,00	**6,00**	,292	,306
		7,00	,035	,308

Table XIV – Test of the similarity between Call type 6, 7 and 8, using PC1 as dependent variable.

a. R Squared = ,021 (Adjusted R Squared = ,015)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6,73[a]	2	3,37	3,76	,024
Intercept	2,76	1	2,76	3,09	,080
Call type	6,73	2	3,37	3,76	,024
Error	319,81	357	,9		
Total	341,37	360			
Corrected Total	326,55	359			

Table XV – Test of the similarity between Call type 6, 7 and 8, using PC2 as dependent variable.

a. R Squared = ,063 (Adjusted R Squared = ,058)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20,37[a]	2	10,19	12,07	,000
Intercept	23,63	1	23,63	27,0	,000
VAR00002	20,37	2	10,19	12,07	,000
Error	301,28	357	,844		
Total	337,12	360			
Corrected Total	321,65	359			

b. Individual signatures

Individuals 1 and 7 exhibited more than one call type per individual: the individual 1 presented three call types (1, 4 and 5) and the individual 7 two call types (1 and 4). The remaining individuals recorded only presented a single call type: call type 4 for the individuals 5 and 6 and call type 1 for the individuals 2 and 3. This difference could be due to sampling. Still the results indicate that individuals can produce more than one call type.

In Table XVI shows that peak frequency is significant (p=0,006) and ascending time is also significant (p=0.002).

Table XVI – Tests of Equality of Group Means for individuals.

	Wilks' Lambda	F	df1	df2	Sig.
Fi	,849	1,11	4	25	,375
Tasc	,518	5,81	4	25	,002
Fasc	,917	,565	4	25	,691
Fmax	,917	,565	4	25	,691
Ffin	,757	2,01	4	25	,124
Pkf1	,571	4,69	4	25	,006
dura	,874	,899	4	25	,479

i. Test for Individual Differences in Call Structure

Four discriminant functions were produced (Table XVII), with only the two first DFs were statistically significant (Table XVIII), with the two explaining 91,9% of the variation. The variables that contributed more to DF1 were ascending time and final frequency, while for DF2 were peak frequency 1, final frequency and duration. The maximum frequency did not enter in any of DFs (Table XIX and XX). The representation of the centroids in both functions (Figure 20) demonstrates that the five individuals have their own signature in the call type 1.

Table XVII – Individuals canonical function statistics.

a. First 4 canonical discriminant functions were used in the analysis.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	2,603[a]	64,0	64,0	,850
2	1,138[a]	28,0	91,9	,730
3	,300[a]	7,4	99,3	,480
4	,028[a]	,7	100,0	,166

Table XVIII – Statistical significance of the canonical function (Wilks' Lambda).

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 4	,097	54,804	24	,000
2 through 4	,350	24,680	15	,054
3 through 4	,748	6,823	8	,556
4	,972	,659	3	,883

Table XIX – Correlations between discriminating variables and standardized canonical discriminant functions.

Variables ordered by absolute size of correlation within function.

*. Largest absolute correlation between each variable and any discriminant function

b. This variable was not used in the analysis.

	Function			
	1	**2**	**3**	**4**
Tasc	,570*	-,242	-,232	,176
Pkf1	,031	,806*	,005	-,577
Fi	,041	,311	,452*	,224
Ffin	-,165	,431	-,303	,647*
dura	-,048	-,312	,285	-,321*
Fasc	,180	,060	-,038	-,220*
Fmax[b]	,180	,060	-,038	-,220*

Table XX – Centroids of the individuals under analysis.

Indiv	Function			
	1	2	3	4
1	-1,585	,060	-,349	,091
2	1,605	-1,461	-,335	-,052
3	-1,001	-,809	1,155	-,018
4	1,524	1,145	,159	,068
7	-,980	1,119	-,197	-,535

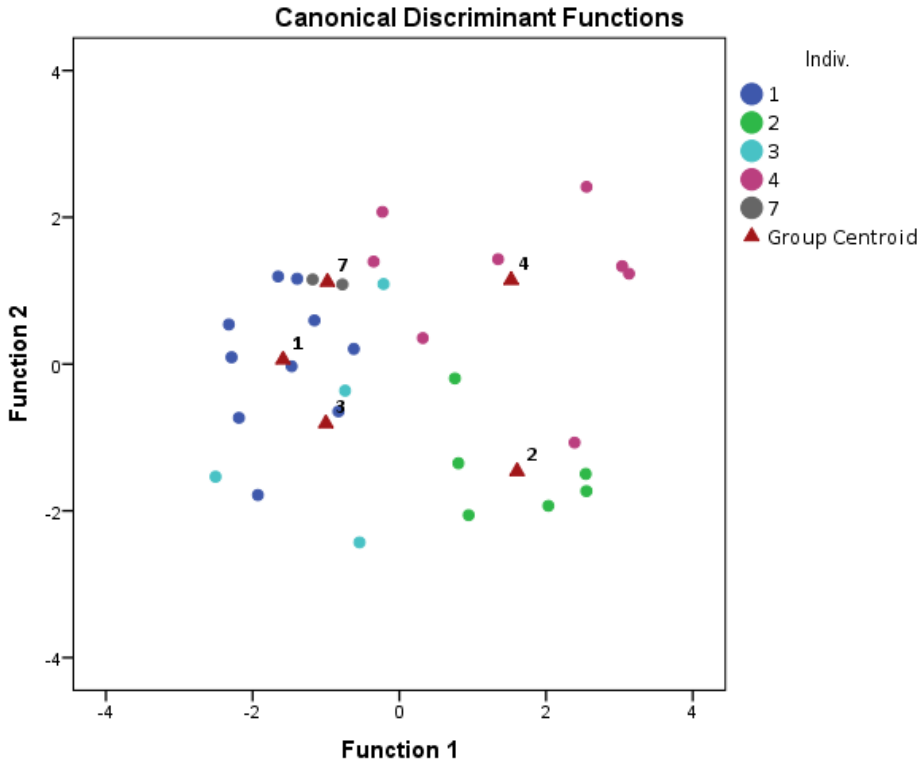

Figure 20 – Representation of the individuals considering their centroids in functions 1 and 2.

The proportion of calls that were correctly classified (Table XXI) was 66,7% for the cases of the original group and 40% for the cross-validated testing. In the original group individual 1 had 7 of the 10 calls (70%) correctly classified, individual 2 was 100%, individuals 3, 4 and 7 were 50% correctly classified. In cross-validated, individual 1 was 40%, individuals 2 and 4 were 50%, individual 3 was 25% correctly classified, while individual 7 was not classified.

Table XXI - Classification Results[a] for the individuals.

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

		Indi	Predicted Group Membership					Total
			1	**2**	**3**	**4**	**7**	
Original	**Count**	**1**	**7**	0	1	0	2	10
		2	0	**6**	0	0	0	6
		3	1	0	**2**	0	1	4
		4	0	1	0	**4**	3	8
		7	1	0	0	0	**1**	2
	%	**1**	**70,0**	,0	10,0	,0	20,0	100,0
		2	,0	**100,0**	,0	,0	,0	100,0
		3	25,0	,0	**50,0**	,0	25,0	100,0
		4	,0	12,5	,0	**50,0**	37,5	100,0
		7	50,0	,0	,0	,0	**50,0**	100,0
Cross-validated[a]	**Count**	**1**	**4**	0	2	0	4	10
		2	0	**3**	1	2	0	6
		3	1	1	**1**	0	1	4
		4	1	1	1	**4**	1	8
		7	2	0	0	0	**0**	2
	%	**1**	**40,0**	,0	20,0	,0	40,0	100,0
		2	,0	**50,0**	16,7	33,3	,0	100,0
		3	25,0	25,0	**25,0**	,0	25,0	100,0
		4	12,5	12,5	12,5	**50,0**	12,5	100,0
		7	100,0	,0	,0	,0	**,0**	100,0

IV | DISCUSSION

In this study, the vocal repertoire of the last two resident Red-Billed Chough (*Pyrrhocorax pyrrhocorax*) populations in Portugal (Serra d'Aire e Candeeiros and Sagres) was investigated. The results show significant variability on the repertoire of the two populations. Were classified 9 different call types, where call type 1 was the most used call type in Serra d'Aire e Candeeiros while in Sagres the most used call type was call type 6. The repertoire from the Serra d'Aire e Candeeiros population was larger and more diverse than the repertoire from the Sagres population. In general, the main differences between both populations on the repertoire are the duration, the maximum frequency and the final frequency. The population from Serra d'Aire has a higher duration and maximum frequency but a lower final frequency than the Sagres population. After the call types statistical analysis, it became evident that both population do not share a common repertoire. The results for the individual calls demonstrated that one individual can produce more than one call type, and that every individual has its vocal signature being the main differences between individuals the ascending time and peak frequency.

4.1. Vocal Repertoire of the populations

It is known the existence of variations in the Chough's repertoire, some peculiar calls in geographically isolated populations were recorded in China, Canaries and Ethiopia (Laiolo et al., 2001). In birds, geographical variations on vocal repertoires may occur in isolated populations or between neighbouring groups (Krebs and Kroodsma, 1980). In comparison with other Iberian populations, including the Serra d'Aire e Candeeiros population, the choughs' population from Sagres is the most genetically isolated (Morinha et al, 2017b) and is also the one with a smaller repertoire (Laiolo et al., 2001). The main differences registered in both studied populations are the duration of the call, the maximum frequency and the final frequency. The population from Serra d'Aire e Candeeiros Natural Park has a higher duration and a higher maximum frequency, however the final frequency is lower than the population from Sagres.

Nevertheless, different studies people have particular criteria for call types classification taking into consideration the geographical contexts under study and, consequently, potential different interpretations in the respective analysis (Cramp and

Perrins, 1994; Laiolo et al., 2000). For example, Sitasuwan and Thaler (1985) have described a total of 14 different call types for the species repertoire. On the other hand, Laiolo et al. (2000) had classified eight different call types from the Scotland populations and several more in a study from populations from all around Europe, Africa and China (Laiolo et al 2001). In our study, nine different call types were identified, five types from population of Serra d'Aire and four from population of Sagres. This variability in numbers of call types can also be justified by the scale range of the studies. In fact, Laiolo et al. (2001) analysed several continuous macro-populations of Chough, where all call types from many different sub-populations were compiled but not differentiated at a population scale. In contrast, in our study the analysis focuses on two isolated micro-populations, from which the respective repertoires that was classified separately and compared between populations. Throughout the analysis it was verified that both populations have different vocal repertoires, which means that non-call types that were from population of Serra d'Aire e Candeeiros was found on the population from Sagres. Even the most common call, here classified as call type 1 and call type 6, that exhibit a similar shape when visually analysed in the spectrogram, are composed by variables statistically different from each other. Despite this difference is not known to what extent the repertoire from each population is implicated on the apparent social segregation suggested by the divergence in the landscape genetic structure between different populations, even without geographical barriers (Morinha et al., 2017b). The differences between the vocal repertoire can also be seen by using the discriminant function analysis (DFA), because it was possible to verify that the two populations have distinctive elements in their vocal repertoire even if we do not consider the call types. In Sagres there was registered call type 9 that was only recorded one time, this call could have been produced by a young bird but we do not know if this possibility is true and for that reason we also describe it.

The main differences can indicate the vocal repertoire is transmitted by learning and not totally by the genetic structure, because the birds are socially isolated, especially during the learning time, all the studies known about the vocal repertoire of the Red-billed Chough do not analysed the geographical variations between populations that are close geographically (Laiolo et al., 2001; Morinha et al., 2017a; Riesch et al., 2006). Even though

it is a new matter in this species there are other studies that demonstrated the same differences between groups in other birds species as is the case of the Long-tailed Tit (Sharp and Hatchwell, 2006), the Apostlebird (Warrington et al., 2015) and Chestnut-crowned Babblers (Crane et al., 2015) and also in mammals as is case of the Killer Whales (Riesch et al., 2006).

4.2. Individuals vocal signatures

The number of individuals recorded was low and the recording effort limited, with just a few recordings from each individual, was limited for testing the existence of potential individual vocal signatures. This was due to restrictions of time and to the very silent behaviour of birds in the vicinity of their nests, producing very few calls per visit. Still, some conclusions can be drawn from the data available.

No detailed studies exist on populations of red-billed Chough that allow to determine how many different call types exist within each population. Still, there is an indication by Cramps and Perris (1998) that birds may produce more than one type of call. Our study shows that each individual produces more than one call type. Indeed, we show that individuals can produce at least three call types. We have no data to confirm that this is common to all individuals from the population, but the possibility that every individual can produce all types of calls is high.

It was also possible in our study to understand that every individual has its own signature even when producing the same call. The characteristics that provide individual identity in sounds are different from those that differentiate sounds, in the same way as two persons can pronounce the same word (ex: beautiful), but the specific components of the sound allow us to reveal their individuality. Because of that, we decided to consider for this analysis all defined variables. We could not consider the variables of the harmonics, due to the quality of the recordings, and they can be of relevance for this individuality identification. Even so, we managed to determine elements of individual signature in the most common call type in the population of Serra d'Aire e Candeeiros. Individuals were mainly distinguished by the ascending time and peak frequency of the call. It would be interesting to have sampled more individuals and for longer, but we are confident that the

results provide important clues for the possibility of individual identification. Performance of playback studies would be the next step for a confirmation of the recognition.

Red-billed Chough have a high social behaviour (Blando et al., 1998) this signature may indicate that the birds have the capacity to distinguish the other individuals from the population has it was found in other corvids (Kondo et al., 2010b).

4.3. Vocal Repertoire and Species Conservation

The Red-billed Chough is distributed all around the Palearctic but, despite its large distribution, the local populations are frequently small and a decreasing of the population abundances due to habitat changes has been registered (Blanco et al., 1998**;** Mourinha, 2017). Considering the species European distribution range, the two chough populations studied have a peripheral location with high isolation patterns as revealed by their low genetic diversity and differentiation levels when compared with the other main chough populations across the Iberian Peninsula (Morinha et al., 2017b). This habitat changes represent specific environmental pressures and ecological constraints, which may alters the behavioural response of the individuals from isolated populations, with potential consequences on the vocal repertoire variability between populations (Krebs and Kroodzman, 1980).

The variability on the repertoire of a species can reflect their genetic structure (Krebs and Kroodzman, 1980). Morinha et al. (2017b) described a possible gene-flow restriction on the Portuguese populations that could not be explain by geographical isolation due to the high level of dispersion of the species and it was suggested then social segregation based on bird communication different patterns. In our study, it was demonstrated that the two populations seemed to be vocally separated, despite some similarities at the species level but statistically different at the population level. In fact, the repertoire changes can occur due to several constraints, from geographical barriers – which is not verified in the in the populations of Red-billed Chough in Portugal (Morinha et al., 2017b) – to the prevailing level of sound in the habitats (windy locations, anthropogenic sound, among others) (Krebs and Kroodzman, 1980).

The annex I of the European Union Council Directive for the conservation of the wild birds says that Red-billed Chough need specials conservation actions in relation to its habitat, because the species is considered as an ecological indicator of the land use changes, i.e., the species are highly dependent on the habitat suitability provided by the traditional farming and pasture for feeding and, therefore, any drastic changes, such as the marked abandonment of traditional rural areas, may induce population decline or local extinction (Blando et al (1998); Morinha et al 2017).

Habitat fragmentation and/or loss may restrict ecological resources for birds, thus influencing behaviour, genetic diversity and fitness of individuals and populations (Keyghobadi, 2007). Considering the ongoing habitat loss and the evidence, that the vocal repertoires divergence of the two main populations in Portugal corroborates the genetic isolation, the understanding of these phenomena is mandatory to develop effective management and preventive conservation strategies in a near future.

4.4. Conclusions and Future perspectives

The results of this study suggest that the individuals can produce more than one call type and have its own signature calls which may indicate that the birds can recognized other individuals by that signature, like already described for other corvids (Kondo et al., 2010b).

At the populations level, the behavioural hypothesis proposed by Morinha et al., (2017b) to explain the genetic isolation observed in Portugal can be confirmed by the results of this study, which reveals clear differences between the vocal repertoires from the two populations under study.

In future research, the analysis of long-term population-occurrence patterns under more representative landscape contexts is a big challenge to improve conservationist management strategies, namely by anticipating future relevant ecological consequences associated with LU/LC changes (de Groot et al. 2010). In this perspective, it is important to increase population sampling in order to establish cause-effect relationships between vocal repertoires and genetic diversity, not only in this two populations but also including other populations in Iberian Peninsula. I also suggest enlarging the sample time, i.e., at least

record the birds on every phenological season and, in other to correctly identify the individuals, it would be important to ring the individuals with color rings for identification from a distance. Also, I think it would be very interesting to perform playbacks of the calls from one population on the other population and recorded the reaction of the individuals.

Although more research is needed in order to achieve accurate conclusions about the ecological meaning of the vocal repertoire variability among Red-billed Chough populations, our study represents a useful contribution through an improved knowledge about the chough repertoire variability under very complex and variable local LU/LC dynamics.

REFERENCES

Banda, E., & Blanco, G. (2014). Strict mate fidelity and reduced breeding dispersal of widowed Red-billed Choughs *Pyrrhocorax pyrrhocorax*. *Bird Study*, *61*(3), 371–377.

Blanco, G., Fargallo, J.A., Tella, J., Cuevas, J.A. (1997). Role of buildings as nest-sites in the range expansion and conservation of choughs *Pyrrhocorax pyrrhocorax* in Spain. Biol. Conserv. 79, 117–122.

Blanco, G., Tella, J. L., & Torre, I. (1998). Traditional farming and key foraging habitats for chough *Pyrrhocorax pyrrhocorax* conservation in a Spanish pseudosteppe landscape. *Journal of Applied Ecology*, *35*, 232–239.

Bluff, L. A., Kacelnik, A., & Rutz, C. (2010). Vocal culture in New Caledonian crows *Corvus moneduloides*. *Biological Journal of the Linnean Society*, *101*(767–776).

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication* (Sinauer As). Sunderland.

Cabral, M. J., J. Almeida, P. R., Almeida, T., Delliger, N., Ferrand de Almeida, M.E. Oliveira, J. M., Palmeirim, A. I., Queirós, C., Rogado, M., Santos-Reis. (2005). *Livro Vermelho dos Vertebrados de Portugal* (Instituto). Lisboa.

Catchpole, C. K., & Slater, P. J. B. (1995). *Bird Song - Biological Themes and Variations* (Cambridge).

Cerqueira, Y. R. (2014). *Social-ecology of rural abandonment : from farmers' perceptions to ecosystem services.* Doctoral dissertation. Faculdade de Ciências. Universidade do Porto. 220pp.

Cramp, S., & Perrins, C. M. (1994). *Handbook of the birds of Europe, the Middle East and North Africa - the birds of the Western Paleartic.* (Oxford Uni). Oxford.

Crane, J. M. S., Pick, J. L., Tribe, A. J., Russell, A. F., & Hatchwell, B. J. (2015). Chestnut-crowned babblers show affinity for calls of removed group members: a dual playback without expectancy violation. *Animal Behaviour*, *104*, 51–57.~

de Groot, R.S., Alkemade, R., Braat, L., Hein, L., Willemen, L. (2010). Challenges in integrating the concept of ecosystem services and values in landscape planning, management and decision making. Ecol.Complexity 7, 260-272.

Elith, J., H. Graham, C., P. Anderson, R., Dudík, M., Ferrier, S., Guisan, A., J. Hijmans, R., Huettmann, F., R. Leathwick, J., Lehmann, A., Li, J., G. Lohmann, L., A. Loiselle, B.,

Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McC. M. Overton, J., Townsend Peterson, A., J. Phillips, S., Richardson, K., Scachetti-Pereira, R., E. Schapire, R., Soberón, J., Williams, S., S. Wisz, M. and E. Zimmermann, N. (2006). *Novel methods improve prediction of species' distributions from occurrence data.* Ecography, 29: 129–151.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Snyder, P.K. (2005). Global consequences of land use. Science, 309, 570–574.

Fontúrbel, F. E. (2013). *Effects of habitat degradation on the monito del monte (Dromiciops gliroides Thomas) and its consequences on the seed dispersal interaction with an endemic mistletoe*. Universidad de Chile. 21pp.

Giery, S. T., Layman, C. A., & Langerhans, R. B. (2015). Anthropogenic ecosystem fragmentation drives shared and unique patterns of sexual signal divergence among three species of Bahamian mosquitofish. *Evolutionary Applications*, *8*, 679–691.

Hoffmann, A. A., & Willi, Y. (2008). Detecting genetic responses to environmental change. *Nature Reviews*, *9*(June), 421–433.

Kaplan, G. (2014). Animal communication. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(6), 661–677.

Keyghobadi, N. (2007). The genetic implications of habitat fragmentation for animals. Can. J. Zool. 85, 1049-1064.

Kondo, N., Watanabe, S., & Izawa, E.-I. (2010a). A temporal rule in vocal exchange among Large-billed Crows (*Corvus macrorhynchos*) in Japan. *Ornithological Science*, *9*(83–91).

Kondo, N., Izawa, E.-I., & Watanabe, S. (2010b). Perceptual mechanism for vocal individual recognition in jungle crows (*Corvus macrorhynchos*) contact call signature and discrimination. *Behaviour*, *147*, 1051–1072.

Krebs, J. R., & Kroodsma, D. E. (1980). *Repertoires and Geographical Variation in Bird Song*. *Advances in Study Behavior* (Vol. II).

Laiolo, P., Palestrini, C., & Rolando, A. (2000). A study of Choughs' vocal repertoire: variability related to individuals, sexes and ages. *Journal of Ornithology*, *141*, 168–179.

Laiolo, P., Rolando, A., Delestrade, A., & De Sanctis, A. (2001). Geographic diversification in

the call repertoire of genus *Pyrrhocorax* (Corvidae). *Canadian Journal of Zoology*, *79*, 1568–1576.

Loureiro, S., Icely, J., & Newton, A. (2008). Enrichment experiments and primary productuin at Sagres (SW Portugal). *Journal of Experimental Marine Biology and Ecology*, *359*, 118–125.

Morinha F., Bastos R., Carvalho D., Travassos P., Santos M., Blanco G., Bastos E. & Cabral J. A. (2017a) A spatially-explicit dynamic modelling framework to assess habitat suitability for endangered species: the case of Red-billed Chough under land use change scenarios in Portugal. Biological Conservation 210: 96-106.

Morinha, F., Dávila, J. A., Bastos, E., Cabral, J. A., Frías, Ó., González, J. L., Travassos, P., Carvalho, D., Borja M., Blanco, G. (2017b). Extreme genetic structure in a social bird species despite high dispersal capacity. *Molecular Ecology*, *26*, 2812–2825.

Newbold, T., Hudson, L.N., Hill., S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger L., Bennett D.J., Choimes A., Collen B., Day J., De Palma A., Díaz S., Echeverria-Londoño S., Edgar M.J., Feldman A., Garon M., Harrison M.L., Alhusseini T., Ingram D.J., Itescu Y., Kattge J., Kemp V., Kirkpatrick L., Kleyer M., Correia D.L., Martin C.D., Meiri S., Novosolov M., Pan Y., Phillips H.R., Purves D.W., Robinson A., Simpson J., Tuck S.L., Weiher E., White H.J., Ewers R.M., Mace G.M., Scharlemann J.P., Purvis A. (2015). Global effects of land use on local terrestrial biodiversity. Nature 520, 45–50.

Oliveira, L. (Dir). (1997a). Nova Encipclopédia Larousse. Vol. *1* (p. 314). Círculo de Leitores. Lisboa.

Oliveira, L. (Dir). (1997b). Nova Enciclopédia Larrousse. Vol. *20* (p. 6163-6333). Círculo de Leitores. Lisboa

Riesch, R., Ford, J. K. B., & Thomsen, F. (2006). Stability and group specificity of stereotyped whistles in resident killer whales, *Orcinus orca*, off British Columbia. *Animal Behaviour*, *71*, 79–91.

Rosa, G. L. M., Ellis, J. M., BBonaccorso, E., & Anjos, L. dos. (16AD). Friend or foe? Social system influences the allocation of signals across functional categories in the repertoires of New World jays. *Behaviour2*, 1–58.

Rufino, R. (Coord. . (1989). *Atlas das Aves que Nidificam em Portugal Continental* (SNPRCN).

Lisboa.

Sharp, S. P., & Hatchwell, B. J. (2006). Development of family specific contact calls in the Long-tailed Tit Aegithalos caudatus. *Ibis*, *148*, 649–656.

Sitasuwa, N., & Thaler, E. (1985). Lautinventar und Verständigung bei Alpenkrähe (Pyrrhocorax pyrrhocorax), Alpendohle (Pyrrhocorax graculus) un deren Hybriden. *Journal of Ornithology*, *126*, 181–193.

Stratford, J. A., & Stouffer, P. C. (1999). Local extinctions of terrestrial insectivorous birds in a fragmented landscape near Manaus, Brazil. *Conservation Biology1*, *13*, 1416–1423.

Titeux, N., Henle, K., Mihoub, J.-B., Regos, A.N., Geijzendorffer, I.R., Cramer, W., Verburg, P.H., Brotons, L. (2016). Biodiversity scenarios neglect future land-use changes. Glob. Change Biol., 22, 2505–2515.

Turner, B. L. I., Lambin, E. F. and Reenberg, A. (2007). The emergence of land change science for global environmental change and sustainability. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 20666- 20671.

Warrington, M. H., Mcdonald, P. G., & Griffith, S. C. (2015). Within-group vocal differentiation of individuals in the cooperatively breeding apostlebird, *26*(2), 493–501.

Wenzel, M. A., Webster, L. M. I., Blanco, G., Burgess, M. D., Kerbiriou, C., Segelbacher, G., Piertney, S.B., Reid, J. M. (2012). Pronounced genetic structure and low genetic diversity in European red-billed chough (*Pyrrhocorax pyrrhocorax*) populations. *Conservation Genetics*, *13*, 1213–1230.

Wong, B. B. M. (2014). Animal communication in a human-dominated world : a comment on Radford et al. *Candolin*, 1033.